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An anatomical and endoscopic study of the nasopharynx and larynx of the donkey (*Equus asinus*)

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INTRODUCTION

The domestic horse (*Equus caballus*) and the donkey (*Equus asinus*) share a common ancestry but have evolved into morphologically distinct species. Interbreeding is possible but the resulting hybrids (mules and hinnies) are usually infertile. In the western world, horses are used extensively for pleasure and sporting pursuits and, since diseases of the respiratory system have an adverse effect on performance, the anatomy and pathophysiology of the respiratory tract have been the subject of extensive research. The gross anatomy of the pharynx and larynx has been described in detail (Cook, 1966; Hare, 1975; Nickel, Schummer & Seiferle, 1979). By contrast, donkeys are used mainly as work animals and play an important role in the economy of the developing countries, but surprisingly little information is available relating to the structure and functional anatomy of the upper airways of this species. Early anatomical works by Boucher (1892) and Lesbre (1923) indicated that there were consistent variations in the respiratory tracts of the two species. Therefore, a study was undertaken to investigate more precisely the anatomical features of the nasopharynx and larynx of the donkey with particular emphasis on those features which are relevant to vocalisation. In this paper the results are described together with some preliminary observations on vocalisation.

MATERIALS AND METHODS

Head and neck specimens from ten donkeys were available for necropsy examination. From five specimens, the larynx and proximal trachea were removed intact through a midline incision on the ventral aspect of the neck for dissection of the laryngeal muscles and mucosa. The remaining five specimens were sectioned using a bandsaw. Four were sectioned in the sagittal plane: one was cut transversely into serial sections 5·0 cm thick from the medial canthus of the eye to the level of the first cervical vertebra. The resulting longitudinal and transverse sections were used to examine the topography, structure and anatomical relationships of the pharynx and larynx. A similar number of horse and pony specimens, of various sizes and ages, was prepared and studied in the same manner. On the median sections, the relative proportions of the rostrocaudal diameters of the nasopharyngeal lumen were measured at three levels parallel to the ventral margin of the vomer: (*a*) at its dorsal part from the junction of the nasal septum with the hard palate to the rostral and caudal margins of the pharyngeal recess; (*b*) in its middle part at the ventral margins

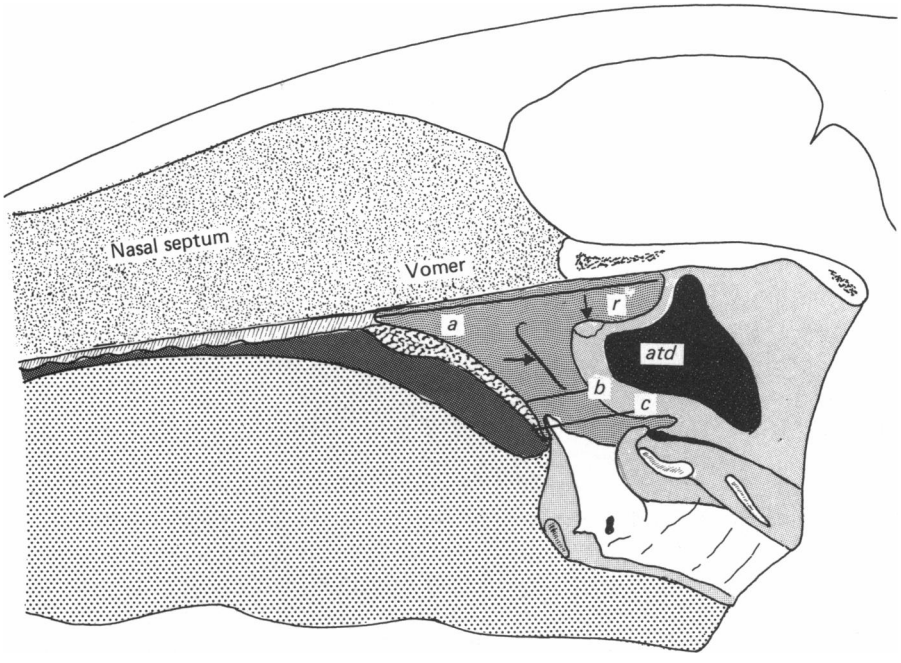

Fig. 1. Diagram of a median section through the head of a donkey to show the three levels (*a*, *b*, *c*) at which the rostrocaudal dimensions of the nasopharynx were measured. Vertical arrow, opening of pharyngeal recess (*r*); *atd*, auditory tube diverticulum; horizontal arrow, auditory tube opening.

of the auditory tube openings; (*c*) ventrally at the pharyngeal ostium (Fig. 1). On median sections of the head and neck of two ponies and two donkeys the angle of the aditus laryngis to a line perpendicular to the basis cranii was measured.

Segments from the wall of the laryngeal saccules and median ventricles of a donkey larynx were taken for histological examination. After fixation in Bouin's solution, alternate serial sections were cut and stained with haematoxylin and eosin, periodic acid–Schiff (PAS) and Masson's trichrome.

Endoscopic examinations of the upper respiratory tract were performed in six donkeys and in ten ponies using flexible fibre optic paediatric gastroscopes (Olympus GIF Type P2, Keymed, Southend, England; Pentax FG 28 B, South Harrow, Middlesex, England). The animals were restrained in stocks and examined without chemical restraint. During endoscopic examination a tape recording of a braying donkey was played to encourage vocalisation. Photographic records in the form of still pictures and ciné films were made. To determine the direction and angulation of the upper airways *in vivo*, radiographs were taken using an Elena–Schonander Triplex Optimatic 1023 unit (Elena–Schonander, Stockholm, Sweden). A pony and a donkey of approximately equal heights were selected and radiographed in the standing position to preserve the normal anatomical relationships between the nasopharynx, the larynx and the trachea.

RESULTS

Necropsy studies

Characteristic of the equine pharyngeal region was the presence of the auditory tube diverticula or guttural pouches, extending caudoventrally from the auditory

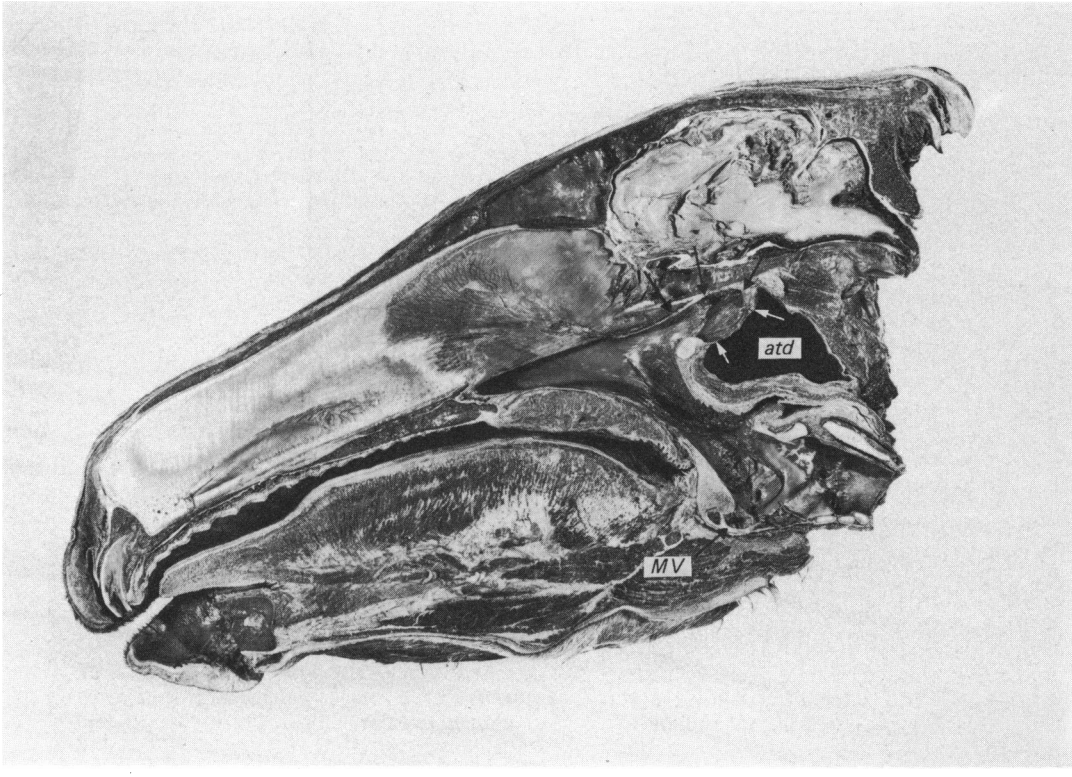

Fig. 2. Median section through the head of a donkey. The arrows indicate the extent of the mucosal pharyngeal recess. *atd*, auditory tube diverticulum; *MV*, median ventricle.

tubes between the base of the cranium and the roof of the nasopharynx (Fig. 2). Each auditory tube diverticulum had a membranous wall, and right and left diverticula were separated by a median septum formed by their medial laminae. Dorsal to the torus tubarius, between the auditory tube openings, there was a diverticulum of the pharyngeal mucosa, the pharyngeal recess. In the donkey, the recess formed a deep membranous pouch, 2·0–3·0 cm in diameter and extending caudally for a distance of 4·0–6·0 cm to the level of the muscular tubercles at the spheno-occipital junction (Fig. 2). Dorsally, the walls of the recess were adherent to the sphenoidal periosteum: caudoventrally, it separated and fused with the medial laminae of the left and right auditory tube diverticula. Rostrally, it communicated with the nasopharynx through a constricted, round or triangular orifice approximately 1·5 cm in diameter.

The lumen of the nasopharynx in both species was constricted at its middle and expanded in its dorsal part. Inclusive of the pharyngeal recess, the ratios of the rostrocaudal diameters of the nasopharynx measured at its dorsal, middle and ventral levels were 3:1:2 in the horse, and 7:1:3 in the donkey specimens. Exclusive of the pharyngeal recess, the ratios were 2·6:1:2 in the horse and 5:1:3 in the donkey. It was evident therefore that in the donkey the pharyngeal air passageway is much more constricted in its middle part, and flares out more, dorsally and ventrally, than in the horse. The angulation of the aditus laryngis differed in the two species. In the horse the aditus was angulated *rostrally* an average of 2·5° from the perpendicular whereas in the donkey the aditus was tilted, on average, 5·5° caudally (Fig. 3).

Dissection revealed that the aryepiglottic folds rostrocaudally were relatively short

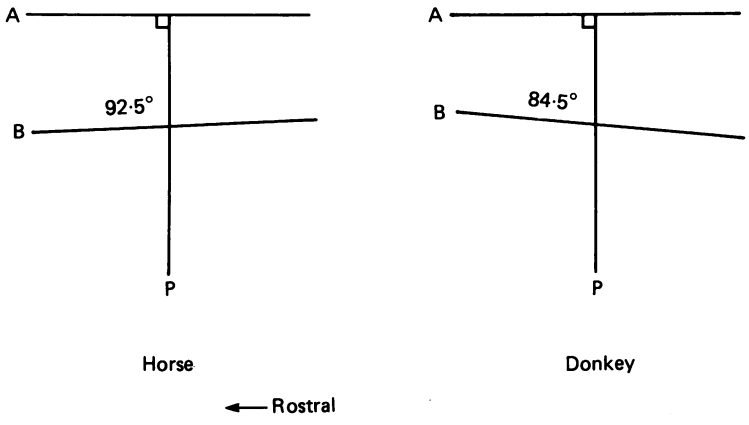

Fig. 3. Comparative angulation of the aditus laryngis to the basis cranii in the horse and the donkey. A, basis cranii; P, line drawn perpendicular to basis cranii; B, frontal plane of the aditus laryngis from the summit of the epiglottic apex to the corniculate cartilages.

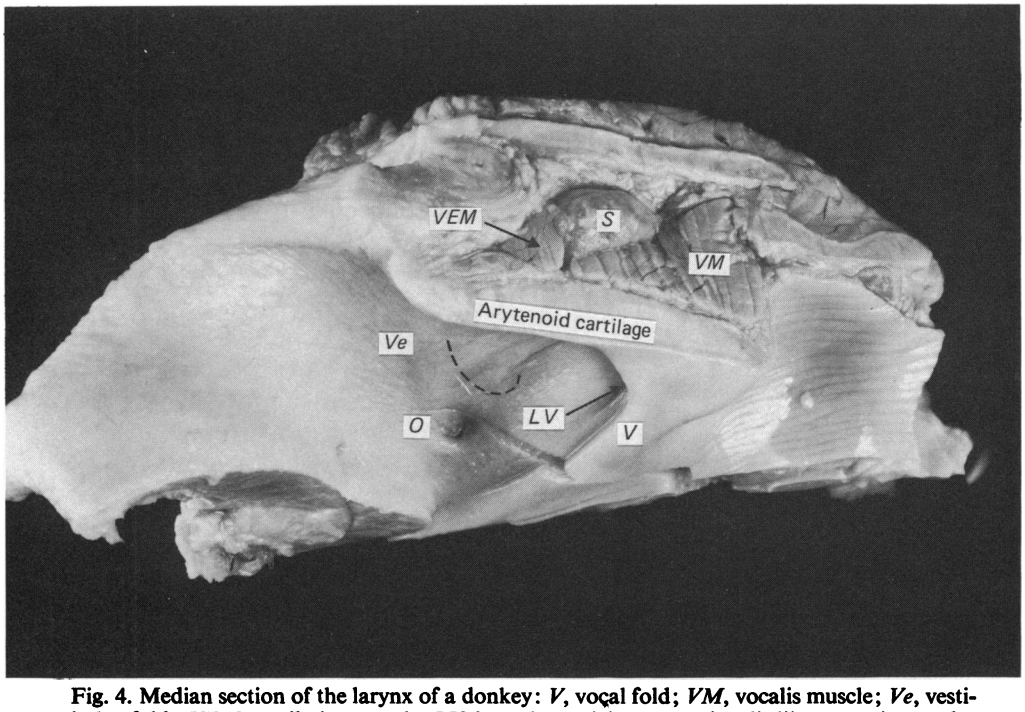

Fig. 4. Median section of the larynx of a donkey: *V*, vocal fold; *VM*, vocalis muscle; *Ve*, vestibular fold; *VEM*, vestibular muscle. *LV*, lateral ventricle: arrow, its slit-like extension under the vocal fold; *S*, dorsal extremity of laryngeal saccule; *O*, opening of laryngeal saccule.

in the donkey and that the apex of the epiglottis was pulled nearer to the arytenoid cartilages. Consequently, with the epiglottis in its normal resting position, i.e. opposed to the caudal border of the soft palate, the aditus faced more caudally in the donkey than in the horse. This anatomical arrangement was consistent with the differences in the angulation of the airway recorded above. The thyroarytenoid muscle of the donkey formed a large, compound muscle mass, interposed between the laryngeal vestibular fold, the lateral ventricle, the vocal process of the arytenoid

cartilage and the vocal fold on its medial side and a laryngeal saccule laterally. The thyroarytenoid muscle was clearly divided into a smaller vestibularis muscle and a larger vocalis muscle (Fig. 4). The vestibularis muscle was related to the rostromedial wall of the laryngeal saccule and more ventrally to the lateral surface of the cuneiform process. The wedge shaped vocalis muscle was interposed between the caudomedial surface of the saccule and the lateral ventricle and vocal fold. An oblique septum divided the muscle into a rostral part which was closely related to the saccule and was partially overlapped medially by the larger caudal part. Vocal and vestibular folds were present in the donkey larynx. The vocal fold measured 3·5–4·0 cm in length, with a well defined, concave rostral margin (Fig. 4). The vestibular fold was a short (1·5–1·8 cm in length), inconspicuous ridge, flattened against the laryngeal wall (Fig. 4). The lateral ventricle was a broad shallow thumb shaped depression bounded by the vestibular fold and the cuneiform process of the epiglottic cartilage rostrally, the vocal fold caudally and the vocal process of the arytenoid cartilage dorsally, lateral to which it formed a shallow slit-like extension (Fig. 4). Near the floor of the larynx at the rostroventral margin of the vestibular fold, a circular orifice led to a membranous cul-de-sac or laryngeal saccule interposed between the thyroid cartilage laterally and the vestibularis and vocalis muscles medially (Fig. 4). The saccule extended from near the floor of the larynx almost to the dorsal border of the thyroid cartilage, curving rostrally at its dorsal and ventral extremities: it was enclosed within a fibrous capsule, thicker rostrally where it was attached to the cuneiform process and vestibular ligament. Fasciculi from the vestibularis and vocalis muscles were attached to the medial capsular wall. The lateral wall of the fibrous capsule was attached by a pad of loose adipose connective tissue to the thyroid cartilage.

The laryngeal saccule communicated with the laryngeal vestibule through a short broad duct which coursed obliquely between the cuneiform process and the rostral vocalis muscle to open at its circular laryngeal orifice. Rostrally, the wall of the orifice was fixed to the underlying cuneiform process. Fasciculi of the rostral vocalis muscle attached to the more mobile caudal wall. A pseudostratified ciliated columnar epithelium with many goblet cells lined the saccule. Mucus-secreting glands were especially numerous in the neighbourhood of its laryngeal orifice. Many loose subepithelial aggregations of lymphocytes were present in the same region.

A median ventricle (Fig. 2) communicated with the laryngeal cavity of the donkey through a round orifice about 0·4 cm in diameter, situated on the midline caudal to the base of the epiglottis. It expanded laterally on each side of the midline to form bilateral pouches up to 1·5 cm deep, between the basihyoid bone and the thyroid cartilage. Its mucosa was thrown into numerous folds lined by stratified squamous epithelium with mucus-secreting glands.

In vivo studies

On endoscopic examination of the donkey nasopharynx, the pharyngeal recess is seen as a dark area, caudodorsal to the openings of the auditory tubes, and few details of the interior were visible (Figs. 5, 6). During quiet respiration, the endoscope could be passed into the recess to visualise its vascular mucous membrane. In response to pressure changes in the nasopharynx, this lining changed its position. During forced inspiration and during the initial high-pitched phase of braying, the caudoventral walls of the recess bulged into its lumen and might protrude through the pharyngeal orifice into the nasopharynx (Fig. 7). In some donkeys, there was a

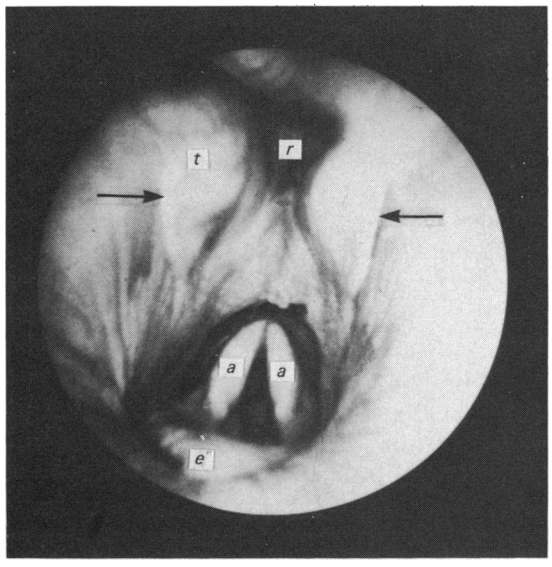

Fig. 5. Endoscopic view of the nasopharynx and larynx of a donkey during quiet respiration: *a*, corniculate processes of arytenoid cartilages; *e*, epiglottis; *t*, torus of auditory tube; arrows, openings of auditory tubes. The recess of the pharyngeal mucosa (*r*) appears as a dark area caudal to the torus of the auditory tubes.

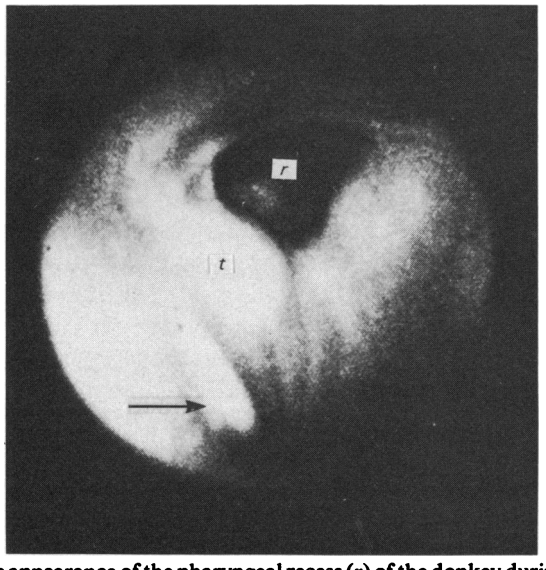

Fig. 6. Endoscopic appearance of the pharyngeal recess (*r*) of the donkey during quiet respiration. Arrow, opening of auditory tube; *t*, torus of auditory tube.

marked asymmetry in these excursions of the walls into the lumen. As the walls of the recess protruded, a concurrent lateral movement of the torus tubarius was seen. Expiration and the low-pitched phase of braying were associated with return of the membrane into the depths of the recess and a medial movement of the torus tubarius to the resting position.

During endoscopic examination of the upper airway of the donkey, the aditus

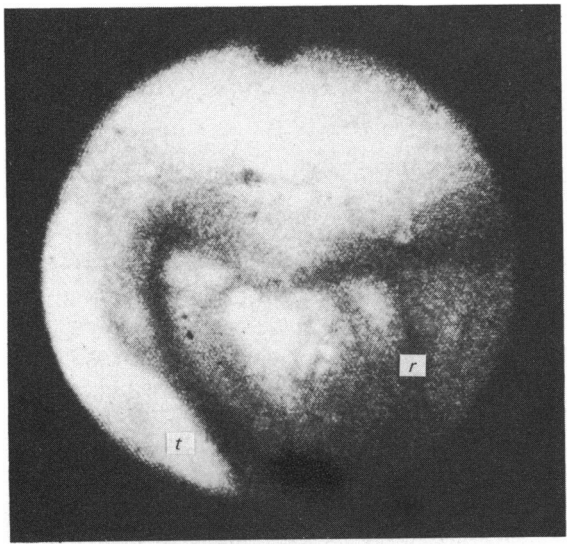

Fig. 7. Endoscopic appearance of the pharyngeal recess (*r*) of a donkey during forced inspiration, with its caudoventral walls protruding into the nasopharynx. *t*, Torus of auditory tube.

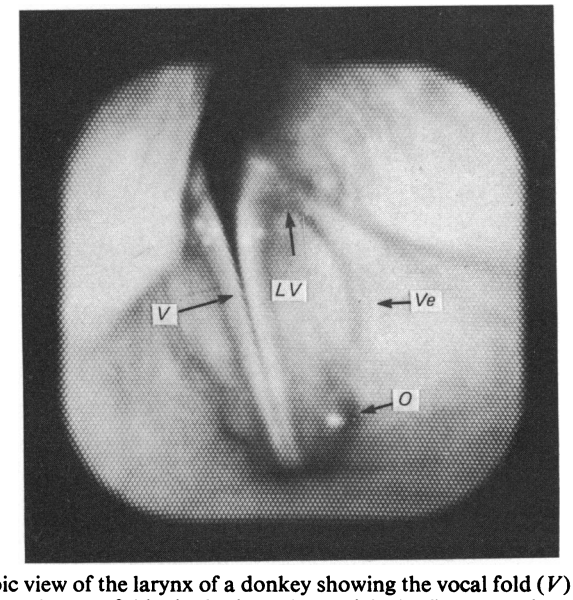

Fig. 8. Endoscopic view of the larynx of a donkey showing the vocal fold (*V*) and the vestibular fold (*Ve*). Between the two folds, is the lateral ventricle (*LV*): arrow, location of its slit-like extension seen in the cadaver. *O*, circular opening leading to the left laryngeal saccule.

laryngis had a more upright orientation than did that of the horse. Radiographs of the upper airways of a donkey and a pony confirmed that the air passageway from the nasopharynx through the larynx was more angulated in the donkey, compared with the relatively straighter alignment in the horse.

Both vocal and vestibular folds of the donkey larynx were clearly defined (Fig. 8). Between the pale, free margin of the vocal fold and the curved edge of the vestibular fold, the broad, shallow lateral ventricle was clearly visible, except when the rima glottidis was fully dilated: its slit-like extension seen in necropsy specimens was not

conspicuous *in vivo*. The openings of the laryngeal saccules were seen near the floor of the larynx ventral to the vestibular folds (Fig. 8).

DISCUSSION

A number of consistent anatomical variations are identifiable in the nasopharynx and larynx of the donkey compared with that of the horse. One of the most obvious differences is in the shape and extent of the pharyngeal recess, which in the donkey is remarkable for its large size and caudal elongation between the left and right auditory tube diverticula. The function of this pouch, if it has a function, is unknown but movements of its membranous walls are observed in response to pressure changes in the upper respiratory tract and the auditory tube diverticula. Inspiration creates a negative pressure in the upper airways, which is resisted by the presence of bone, cartilage and muscle in their walls (Robinson & Sorensen, 1978). Since the ventrolateral walls of the donkey pharyngeal recess have no comparable support, they may tend to be pulled into the pharynx during forced inspiration and to recede during expiration. The pharyngeal recess has been shown to have a close physical relation with the medial laminae and septum of the auditory tube diverticula and concurrent movements of the torus tubarius are associated with excursions of the walls of the recess. It is equally likely, therefore, that the excursions of the recess reflect pressure changes within the auditory diverticula and that the asymmetric movements of its wall are due to differing pressures in the two diverticula.

Little is known about the mechanics of vocalisation in donkeys, but the fact that the pharyngeal recess is seen to bulge into the airway during the high-pitched part of the bray supports the theory that an inspiratory effort accompanies this sound. The lower-pitched part of the bray is accompanied by a return of the membrane into the depths of the recess and is probably expiratory, as are most vocalisations. The morphology of the asinine pharyngeal recess and the movements of its wall during respiration and phonation have not been described previously.

Boucher (1892) reported that the donkey epiglottis is relatively shorter and more sharply pointed than that of the horse, resulting in a reduction of the dorsoventral diameter of the aditus laryngis. Both Boucher (1892) and Lesbre (1923) described the aditus laryngis of the horse as being oval in shape and more elongated than that of the donkey. In the latter species, the transverse diameter is greater relative to both the dorsoventral diameter and the rostrocaudal length. The observations recorded here in the live animal and postmortem specimens support these authors' comments.

In the present study, laryngeal dissections and endoscopic examinations have revealed marked variations in the size and disposition of the laryngeal ventricles between the horse and donkey. The median ventricle is a simple midline depression in the horse, but is expanded into bilateral pouches in the donkey. In the latter species, Lesbre (1923) described the median ventricle as a deep depression at the base of the epiglottis, whereas Boucher (1892) found much individual variation but did not identify any species-dependent variations between the horse and donkey.

In the horse, the lateral laryngeal ventricle communicates with a mucosal recess through a wide orifice. The *Nomina Anatomica Veterinaria* (1973) recommends that the entire structure be called the lateral ventricle. Other authors have retained the term laryngeal saccule to describe the mucosal recess (Schneider, 1964; Dorst, 1973). In contrast to the horse, the donkey has a broad shallow depression between the vestibular and vocal folds and a large mucosal pouch communicating with the

larynx through a separate orifice near the ventral extremity of the vestibular fold. These findings agree with the description given by Lesbre (1923). It is suggested that the large mucosal pouch in the donkey is more accurately termed the laryngeal saccule. The saccule is lined by a respiratory type of epithelium containing numerous mucus-secreting cells. Contraction of the muscle fibres attaching to the fibrous wall of the saccule must control the internal pressures, the patency of its laryngeal orifice and the expulsion of glandular secretion. The presence of lymphoid tissue in the wall of the saccule has not been described previously.

At present the functions of the laryngeal ventricles and saccules of the donkey are speculative but they may affect vocalisation. The bray of the donkey is audibly different to the neigh or whinny of the horse. Braying is a loud, penetrating sound that allows donkeys to locate and communicate with each other over long distances and to distinguish conspecifics from other equidae sharing a common territory under feral conditions. In the present study, braying has been shown to have an inspiratory and an expiratory component, which is unusual since most vocalisations are expiratory in nature. It is well recognised that the angulation of the airway and the shape of the nasopharynx affect the character of resonance during vocalisation, thereby contributing to the quality of the voice. The characteristic resonance of the bray is likely to be related to the morphology of the airway of the donkey. Measurements and radiographs reveal a greater angulation of the airway in the laryngeal region of the donkey than of the horse. Differences have also been recorded in the relative proportions of the diameter of the nasopharyngeal lumen measured at three levels. Although further studies using radiographic techniques would be necessary to assess these proportions in the live animal, measurements in the cadaver show consistent differences between the species.

It is interesting that the audibility of the bray often presents a problem in jungle warfare, necessitating surgical muting operations as a routine procedure in donkeys used by the armed forces (Stewart, 1945). In a description of the ventriculectomy procedure used to devoice mules, Phillips (1959) notes that the larynx of the pony and mule differ in the shape of the 'ventricle' and the position of its opening into the larynx. As a result, the stripping procedure is found to be more difficult in the mule. Although Cuvier (1817) considered "the hoarseness of the voice or bray of *Equus asinus* to depend upon two small peculiar cavities situated at the bottom of the larynx", the mechanics of braying are still not adequately understood but further research is in progress to investigate the movements of the larynx during phonation.

SUMMARY

Using histological techniques, anatomical dissection, fibre optic endoscopy and radiography, the nasopharynx and larynx of the donkey were examined and described. Compared with horses, donkeys have a much deeper pharyngeal recess extending 4·0–6·0 cm caudally from a constricted orifice, through which it communicates with the nasopharynx. Movements of the membranous lining of the recess reflect pressure changes in the upper airways. A shallow, thumb shaped depression on the rostral face of each vocal fold corresponds in position to the lateral ventricle in the horse. In the donkey, large paired laryngeal saccules, interposed between the thyroarytenoideus muscle and the thyroid cartilage, open into the laryngeal cavity through small circular orifices near the rostroventral extremity of the vestibular folds. The large thyroarytenoideus muscle is subdivided into vestibular and vocal parts,

which are thought to control the expression of secretion from the laryngeal saccules. The unusual characteristics of vocalisation in the donkey may be related to the shape and orientation of the resonance chambers of the upper airways. In comparison with the horse, the asinine nasopharynx is markedly constricted in its middle part and the laryngeal airway has a more acute angulation relative to the nasopharynx. Vocalisation in the donkey was observed to have an inspiratory and an expiratory component.

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